

### Claims

1. A method of monitoring progression of a xenograft in a non-human host animal comprising:
  - 5 (i) genetically modifying or engineering a cell before or after implantation into an animal so as to incorporate at least one reporter molecule and/or reporter gene and/or reporter agent into said cell;
  - (ii) implanting said modified cell into said host animal and allowing a xenograft to grow for a sufficient period of time; and
  - 10 (iii) measuring at least one parameter of a selected biochemical/physiological response associated with the reporter molecule or reporter gene.
2. A method according to claim 1 wherein there is a plurality of genetically modified or engineered cells which are human or non-human in origin.
- 15 3. A method according to either preceding claim wherein the cell is a primary isolate derived from normal tissue or a tumour or is an immortalised or established cell line.
- 20 4. A method according to any preceding claim wherein the reporter molecule is selected from the group comprising a protein, an antigen, an enzyme, an enzyme substrate, fluorescent, chemiluminescent, chromogenic agent or radionuclide.
- 25 5. A method according to any preceding claim wherein the reporter gene is selected from those genes encoding proteins chloramphenicol-acetyltransferase, galactosidase, glucuronidase, luciferase, beta-galactosidase or green fluorescent protein, secreted alkaline phosphatase (SEAP), major urinary protein (MUP) or human chorionic gonadotrophin (hCG).

6. A method according to any previous claim where the reporter agent is a protease or kinase or where the reporter is a protein or RNA effecting changes in protein or mRNA stabilisation.
- 5 7. A method according to any preceding claim wherein the host animal is a rodent.
8. A method according to claim 7 wherein the rodent is a mouse or rat.
- 10 9. A method according to either claim 7 or 8 wherein the rodent is a wild type or genetically engineered mouse or rat having a specifically selected genetic background.
- 15 10. A method according to any preceding claim wherein the host animal has more than one different population of reporter cells/system implanted therein.
- 20 11. A method according to any preceding claim wherein the step comprising measuring at least one parameter of a selected biochemical/ physiological response associated with the reporter molecule or reporter gene or reporter agent a qualitative or quantitative measurement and may involve invasive or non-invasive procedures in order to ascertain such data.
- 25 12. A method according to any preceding claim wherein the xenograft is allowed to proliferate as a xenograft tumour with or without metastatic tumours at secondary sites.
- 30 13. A method according to any preceding claim wherein the implanted modified cells are introduced into the host animal either as individual cells suspended in suitable medium or as tumour fragments.

14. A method according to any preceding claim wherein the implanted modified cells are allowed to grow in the host animal either systemically or as a xenograft tumour at the site of implantation.
- 5 15. A method according to any preceding claim wherein the host animal is either immunosuppressed by means of administration of appropriate immunosuppressant agents or is of an immunocompromised strain or is immunologically intact and wherein the implanted modified cells are syngeneic with the host animal.
- 10 16. A method according to any preceding claim wherein the reporter cell/system is genetically engineered to express a transgene or multiple transgenes.
17. A method according to any preceding claim wherein the reporter cell/system expresses the reporter gene(s) or agent(s) at the time of implantation or is transfected  
15 *in vivo* with the reporter gene or agent in a specifically targeted manner.
18. A method according to any preceding claim wherein the reporter gene(s) or agent(s) comprise at least one element that allow measurement of a biochemical parameter in response to either changes in cell physiology occurring during reporter  
20 cell/system proliferation or as a result of toxicological or pharmacological effects of an administered xenobiotic-compound or biological substance.
19. A method according to any preceding claim wherein the step of measuring at least one parameter of a selected biochemical/ physiological response associated with  
25 the reporter molecule or reporter gene or reporter agent comprises measuring or monitoring any one or more of the following parameters:
- (a) reporter cell numbers, cell cycle modulation or mitotic fraction, cell differentiation, angiogenesis, hypoxia, cell death by necrosis, cell lysis or apoptosis;
- (b) oxidative stress, DNA damage, mitochondrial function, membrane  
30 perturbation, GSH depletion, receptor-mediated toxicity, enzyme inhibition, cofactor

availability, pH or osmotic change, perturbation of calcium homeostasis, cell differentiation, protein turnover, ubiquitination or protein misfolding;

(c) effects on intracellular signalling pathways, receptor interactions, effects on gene transcription, translation or protein stability, hormone or growth factor receptor modulation, peroxisome proliferator-activated receptor modulation, intracellular  
5 signal transduction pathways, MAP kinase or phosphatase signalling, p53 signalling or ras signalling; and

(d) induction of drug resistance mechanisms, drug delivery or drug bystander effects.

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20. A method according to any preceding claim wherein the reporter gene comprises a naturally occurring or artificial promoter sequence driving expression of a gene resulting in production of a reporter protein.

15 21. A method according to claim 20 wherein the promoter is constitutively active or is inducible

22. A method according to any preceding claim wherein the reporter gene expression product(s) is/are reportable transcriptionally or post-transcriptionally.

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23. A method according to claim 22 wherein transcriptional reporting is mediated by any one of the gene promoters selected from a group comprising, vascular endothelial growth factor (VEGF), nitric oxide synthetase (iNOS) promoter, haemoxygenase-1 (HO-1) promoter, cyclo-oxygenase-2 (COX-2) promoter, transglutaminase promoter, Peg3/pw1 promoter, 14-3-3 protein promoter or a  
25 GADD153 promoter.

24. A method according to claim 22 wherein post-transcriptional reporting is mediated through generation of a protein that can effect protein modifications, for  
30 example as a consequence of protease activity that result in translocation of a cytoplasmic protein to the nucleus or from membrane-bound form to secreted form or

through protein cleavage or activation of a proenzyme or transcription factor or deactivation of an active enzyme or transcription factor or in secretion into the blood or excretion into urine.

5 25. A method according to claim 22 wherein post-transcriptional reporting is mediated through production of a protein or RNA that effects changes in the stabilisation of a protein or mRNA.

10 26. A method according to claim 22 wherein post-transcriptional reporting is mediated through production of a protein which on death or lysis of the cell expressing it, is secreted or excreted, for instance alanine aminotransferase.

15 27. A method according to any preceding claim wherein measuring of at least one parameter of a selected biochemical/ physiological response associated with the reporter molecule or reporter gene or reporter agent is by means of either a non-invasive or an invasive assay wherein:

(i) the non-invasive assay is in excreted body products, or by bioluminescence measurement, or by blood pressure measurement, or by transcutaneous oxygen tension measurement, or by nuclear magnetic resonance  
20 measurement or by positron emission tomographic measurement; or

(ii) an invasive assay for blood or xenograft reporter products.

25 28. A product produced by the method of claim 1 and comprising a genetically modified or engineered cell, the modification or engineering of the cell being such that the cell comprises at least one reporter molecule or reporter gene.

29. A product according to claim 28 further including any one or more of the features recited in claims 2 to 27.

30. An artificial gene construct comprising a promoter region of the SFN gene and human chorionic gonadotrophin (hCG) such that expression of hCG is controlled by the SFN promoter.
- 5 31. A construct according to claim 30 wherein 5'-regulatory promoter region of the SFN gene is linked to hCG.
32. A human tumour-derived cell line containing the construct of claims 30 or 31.
- 10 33. A non-human animal containing a human tumour-derived cell line according to claim 32.
34. Use of the method of claim 1 or product of claim 28 or 30 in any one or more of the following situations:
- 15 (a) measuring reporter cell proliferation, particularly but not exclusively during xenograft growth or differentiation or death or in response to treatments;
- (b) determining the mechanism of differentiation or death where reporter cell differentiation or death occurs;
- 20 (c) monitoring processes in secondary metastatic tumours where these may differ in their responses from the primary reporter cell xenograft;
- (d) making non-invasive measurements of parameters related to biochemical processes in the reporter cell/system;
- (e) identifying drug-resistant cell populations, for example arising from differential toxicity of a drug to dividing as compared to non-dividing cells or to hypoxic as opposed to normoxic cells;
- 25 (f) determining the effects of genetic background on tumour cell growth or on response to treatments, for instance in cells expressing and not expressing p53;
- 30 (g) making dynamic measurements using reporter molecules or genes of short half lives or that are excreted; and

- 5 (h) determining or confirming the targets of drug action *in vivo*; and  
(i) measuring drug bystander effects; and identifying promoter elements involved in gene regulation; and  
(j) determining intracellular drug concentrations and thereby those cells  
that take up a drug; and  
(k) determining or confirming the targets of drug action *in vivo*.

10 35. A kit comprising a product with at least one reporter cell/system as defined in either claim 28 or 29 and optionally a set of instructions therefore.

36. A kit according to claim 35 wherein the product is supplied as a suspension of reporter cells.

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